

EFFECT OF CHRONIC RESTRAINT ON GASTRONINTESTINAL FUNCTION

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March 1967

FACILITY FORM 802	N67-21424	
	(ACCESSION NUMBER)	(THRU)
	26	
	(PAGES)	(CODE)
	NASA CR-73071	04
	(NASA CR OR TMX OR AD NUMBER)	(CATEGORY)

Prepared under NASA Order A73704 by
 BATTELLE MEMORIAL INSTITUTE
 Pacific N.W. Laboratories

for Ames Research Center

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Effect of Chronic Restraint on Gastrointestinal Tract Function

Since astronauts will be subjected to long periods of near immobility, there is a definite need for information about the influence of such restraint on gastrointestinal function.⁽¹⁾ The objective of the present research was to determine if the prolonged restraint of laboratory animals (the rat) adversely affects absorption of fluid and electrolytes.

The experiments performed may be separated into three categories: The first was to determine the influence of chronic restraint on the transport properties of the rat small intestine for electrolytes and fluid, both in vitro and in vivo; the second, to determine if chronic restraint causes latent effects that are manifested by more severe acute restraint; and the third, to determine if the effects of chronic restraint and the known changes in function that occur after exposure to ionizing radiation are additive. Data were also obtained on the influence of chronic restraint on calcium absorption and retention, body, kidney, femur and femur ash weight, and the histopathology of the rat small intestine.

The results obtained from these studies did not demonstrate that chronic restraint has a deleterious effect either on intestinal function or the susceptibility of the small intestine to more severe stress. As previously observed,⁽²⁾ chronic restraint did depress the growth and, in addition, we found that body weight and femur and kidney weights were depressed in the same proportion. However, bone mineralization, as indicated by percent ash to whole femur weight, was perhaps transiently, but not permanently, affected.

I. Influence of Chronic Restraint on Rat Weight

Male, Charles River strain CD rats were confined in restraint cages (Fig. 1) of the same design as those used by Pfeiffer.⁽²⁾

The width of each cage was adjusted according to the weights of the rats, so that lateral movement was restricted to the cage widths shown in Table I. This space allowed the rats to rest in apparent comfort but severely limited movement. Control rats were kept in individual cages, alternated between the cages for the restrained rats. All rats were allowed food and water ad libitum.

Table I. Cage Widths for Chronically Restrained Rats

<u>Rat Weight</u>	<u>Cage Width</u>
100-130 g	4.0 cm
131-182	4.5
183-238	5.0
239-312	5.5
312-400	6.0
400-504	6.5

The data in Table II show that the restraint procedure caused a significant depression in the growth of a group of rats restrained for 10 weeks. Analysis of weekly weight gains (Table III) indicated that the depression in growth was most significant during the first, second, third, sixth and seventh weeks of restraint. Individual

Table II. Influence of Chronic Restraint on Rat Body Weight

<u>Length of Restraint</u>	<u>Body Weights*</u>		<u>Probability of Difference</u>	<u>Number of Rats Control/Restrained</u>
	<u>Control Rats</u>	<u>Restrained Rats</u>		
0 days	156 \pm 2 g	151 \pm 2 g	>0.50	29/23
4	172 \pm 3	160 \pm 3	>0.01	"
11	214 \pm 2	193 \pm 2	"	"
18	267 \pm 4	246 \pm 4	"	"
25	315 \pm 3	284 \pm 5	"	"
29	338 \pm 4	313 \pm 5	"	25/19
36	365 \pm 5	336 \pm 5	"	21/15
42	392 \pm 6	354 \pm 7	"	17/11
49	415 \pm 6	370 \pm 6	"	"
56	435 \pm 7	385 \pm 7	"	"
63	453 \pm 7	401 \pm 7	"	"

*Mean rat weight and standard error of mean. The decrease in the number of rats after the 25th day was due to their use on experiments.

Table III. Influence of Chronic Restraint on Rat Weight Gain

<u>Interval</u>	<u>Weight Gain*</u>		<u>Probability of Difference</u>
	<u>Control Rats</u>	<u>Restrained Rats</u>	
0-4 days	24 \pm 1 g	15 \pm 2 g	>0.01
4-11	36 \pm 2	31 \pm 2	>0.20
11-18	57 \pm 3	46 \pm 5	>0.20
18-25	42 \pm 2	37 \pm 3	>0.50
25-29	24 \pm 3	29 \pm 2	$0.50 <$
29-36	27 \pm 2	24 \pm 2	$0.50 <$
36-42	26 \pm 1	21 \pm 1	>0.05
42-49	22 \pm 1	16 \pm 2	>0.05
49-56	20 \pm 2	15 \pm 2	>0.20
56-63	19 \pm 2	16 \pm 2	>0.20

*Mean weight gain and standard error of mean. All of the values shown are for the 17 control rats and 11 restrained rats kept throughout the 10 weeks shown in Table II.

weight records indicated that the lower average weight gain for the restrained rats during the sixth and seventh weeks was partly caused by transient depressions of weight gain for some of the restrained rats. During these intervals, the rats ate little and their cages were difficult to keep clean; requiring more frequent washings and sterilization.

On two occasions during other experiments, a severe weight loss occurred requiring the autopsy of restrained rats. One of the two autopsies indicated pulmonary infection and the other rat had a salmonella infection. Cultures of fecal samples obtained directly from the other restrained and control rats in that group did not indicate the presence of pathogenic flora.

II. In Situ Perfusion Measurements

Three studies were performed to evaluate the influence of chronic restraint on fluid and electrolyte insorption* by the small intestine, in situ. For the first study, the influence of 3 to 4 weeks chronic restraint on intestinal function was studied by unidirectional pumping (1.36 ml/min) of physiological buffer (pulse labeled with ^{22}Na , ^{36}Cl , ^{45}Ca , ^3HOH and polyethylene glycol) through the small intestine of anesthetized rats, accompanied by frequent sampling of the perfusate. The experimental apparatus and surgical procedures used have been described previously.⁽³⁾

*Insorption = movement of test substance from lumen to blood.

Measurements of insorption were made for six restrained and six control rats during four successive test intervals. As indicated in the experimental design shown in Table IV, the buffered perfusate contained the radioactive labels only during the initial 10 minutes of each test interval. The labeled buffer during the first test interval contained 15 mM glucose; during the second test interval was glucose-free; during the third test interval the Na^+ and Cl^- concentrations were reduced to 50 mM and 20 mM respectively with mannitol substituted for NaCl; and during the final test interval, the normal media with 15 mM glucose was again used.

Table IV. Perfusion Procedure Used for Data Shown in Table V.

<u>Test Interval</u>	<u>Perfusate*</u>	<u>Time Minutes</u>
Conditioning Period	Normal	30
1	Radioactive, 15 mM glucose	10
1	Normal	20
2	Radioactive, without glucose	10
2	Normal	20
3	Radioactive, 15 mM glucose, Na (50 mM), Cl (20 mM)	10
3	Normal	20
4	Radioactive, 15 mM glucose	10
4	Normal	40

*Each buffer was 310 mOsm; normal buffer was not radioactive and was glucose-free, and mannitol and Na_2SO_4 were substituted for NaCl during the third test interval. The calcium concentration was 1 mM.

The results of these measurements are shown in Table V. As shown, chronic restraint had little effect on insorption. Sodium-22, ^{36}Cl , and ^3HOH insorption were depressed by eliminating glucose from the perfusate, but this effect was similar for the restrained and control rats.

Table V. Effect of Three to Four Weeks Restraint on Na, Cl and Water Insorption by the Rat Intestine

<u>Test Interval</u>	<u>Control Rats</u>		
	<u>^3HOH</u>	<u>^{22}Na</u>	<u>^{36}Cl</u>
1	85 \pm 3	47 \pm 3	22 \pm 2
2	83 \pm 4	42 \pm 5	18 \pm 6
3	84 \pm 3	56 \pm 5	37 \pm 3
4	88 \pm 2	48 \pm 2	22 \pm 4
<u>Test Interval</u>	<u>Restrained Rats</u>		
	<u>^3HOH</u>	<u>^{22}Na</u>	<u>^{36}Cl</u>
1	86 \pm 3	47 \pm 5	21 \pm 3
2	80 \pm 2	37 \pm 3	14 \pm 3
3	84 \pm 3	62 \pm 5	42 \pm 5
4	88 \pm 2	50 \pm 3	23 \pm 4

Each value is the mean and standard error for six rats.

Insorption values are expressed as the percent of the perfused radioactive label that was absorbed. Each rat was fasted one day prior to the perfusion measurements. ^{45}Ca insorption was usually less than 10 percent and variable. During restraint the average weights of the restrained rats increased from 351 to 376 grams and for the control rats 354 to 412 grams.

In Table VI are the results of a second study for which glucose-free perfusate labeled with ^{22}Na and ^3HOH was passed through the intestine for two hours. These results, like those in the previous study, did not show a serious influence on the state of absorption from chronic restraint.

Table VI. Absorption of ^3HOH and ^{22}Na from Buffer Infused Through the Small Intestine In Situ for Two Hours

<u>Treatment</u>	^3HOH	^{22}Na
	<u>Absorption</u> <u>% of Administered Dose</u>	<u>Absorption</u> <u>% of Administered Dose</u>
Control	71	42
	67	34
	<u>68</u>	<u>34</u>
	Average	38
5-weeks Restraint	63	30
	62	35
	66	38
	67	38
	66	36
	<u>60</u>	<u>29</u>
	Average	34

Because of the limited, if any, effect from chronic restraint during the initial in situ perfusion studies, another experiment was

performed to determine if chronic restraint causes latent effects on the rat small intestine that would become evident when a second stress was superimposed upon the first. The second stress condition used was to fast the rats for two days followed by severe restraint. The rats were bound tightly with surgical gauze for four hours preceding perfusion. Measurements were made on 12 control and 12 chronically restrained (5-7 weeks) rats. Half of the rats in both the control and restrained groups were subjected to acute restraint and then perfused as before with the exception that labeled buffer was infused during the initial 15 minutes of each test interval. Mannitol buffer was not used. The procedure used is shown in Table VII.

Table VII. Perfusion Procedure Used for
Data Shown in Tables VIII and IX

<u>Test Interval</u>	<u>Perfusate*</u>	<u>Time Minutes</u>
Conditioning Period	Normal	30
1	Radioactive, 15 mM glucose	15
1	Normal	20
2	Radioactive, without glucose	15
2	Normal	20
3	Radioactive, 15 mM glucose	15
3	Normal	40

*Each buffer was 310 mOsm; normal buffer was not radioactive and was glucose-free. During restraint the average weights of the chronically restrained rats increased from 151 to 284 grams and for the control rats from 156 to 315 grams.

The results (Table VIII) obtained for rats that had been restrained 5-7 weeks and unrestrained control rats were comparable, and similar to the results obtained during the first study. Sodium, chloride and tritiated water insorption were significantly depressed in the absence of glucose.

Table VIII. Effect of Five to Seven Weeks Restraint on Na, Cl and Water Insorption by the Rat Small Intestine

<u>Test Interval</u>	<u>Control Rats</u>		
	<u>^3HOH</u>	<u>^{22}Na</u>	<u>^{36}Cl</u>
1	83 \pm 3	50 \pm 3	29 \pm 2
2	77 \pm 1	40 \pm 2	17 \pm 4
3	84 \pm 2	49 \pm 3	25 \pm 4

<u>Test Interval</u>	<u>Chronically Restrained Rats</u>		
	<u>^3HOH</u>	<u>^{22}Na</u>	<u>^{36}Cl</u>
1	83 \pm 1	50 \pm 3	26 \pm 2
2	81 \pm 2	41 \pm 2	19 \pm 2
3	83 \pm 1	45 \pm 2	24 \pm 2

The values for the first and third test intervals are the means and their standard error for six rats; and for the second test interval, five rats. Insorption values are expressed as the percent of the perfused radioactive label that was absorbed. ^{45}Ca insorption was about 10 percent and variable.

As shown in Table IX, acute restraint did not cause a significant difference in the initial insorption values for the chronically restrained and control rats. However, use of the glucose-free media during the second test interval did not cause a significant depression in the insorption of ^{22}Na , ^{36}Cl and ^3HOH . Although the differences were not appreciable the requirement for glucose was more evident in the chronically restrained rat than the control. The insorption values for ^{22}Na , ^{36}Cl and ^3HOH from the glucose-free media for the rats subjected to acute restraint, in addition to chronic restraint, (Table IX), were not significantly greater than those for the control rats run in that experiment. Both results were somewhat higher than the data shown in Table VIII.

Table IX. Effects of Five to Seven Weeks Chronic Restraint Plus Acute Restraint Stress on Na, Cl and Water Insorption by the Rat Small Intestine

<u>Test Interval</u>	<u>Control Rats</u>		
	<u>^3HOH</u>	<u>^{22}Na</u>	<u>^{36}Cl</u>
1	87 \pm 2	52 \pm 2	27 \pm 2
2	85 \pm 2	46 \pm 2	24 \pm 2
3	87 \pm 2	52 \pm 3	26 \pm 2
<u>Test Interval</u>	<u>Chronically Restrained Rats</u>		
	<u>^3HOH</u>	<u>^{22}Na</u>	<u>^{36}Cl</u>
1	85 \pm 3	53 \pm 4	26 \pm 4
2	83 \pm 3	51 \pm 3	27 \pm 3
3	84 \pm 2	52 \pm 3	25 \pm 2

The values for the first and third test intervals are the means and their standard error for six rats, and

for the second test interval, five rats. Insorption values are expressed as the per cent of the perfused radioactive label that was absorbed. ^{45}Ca insorption was about 10 per cent and variable.

III. In Vitro Studies

It was apparent from the in situ perfusion measurements that effects on the net absorptive state of the small intestine from chronic restraint were neither large nor likely to be demonstrated by further studies of this kind. Therefore, in vitro studies were performed to evaluate the effects of chronic restraint on the bioelectric and transport properties of specific anatomical regions of the small intestine. In vitro procedures allow measurement of the ability of specific anatomical regions to actively transport electrolytes together with evaluation of the passive transport characteristics of the intestine. Voltage measurements are dependent upon the permeability characteristics, active ion and sugar transport and enzyme activities in the intestine. The in vitro techniques used for this study have been described previously.⁽⁴⁾

For the first study, voltage measurements were made for intestinal segments from five rats subjected to chronic restraint for two weeks and five control rats. All of the rats were fasted one day and then fed two hours before the measurements. Although the results demonstrated differences between different anatomical regions of the intestine, there

was not a significant effect on the bioelectric potential measurements from chronic restraint. The major results from this study are shown in Figure 2.

IV. Calcium Absorption and Retention

Calcium- ^{45}Ca absorption by rats perfused in situ was low (about 10 percent) and that function was not significantly altered by restraint. Because of the importance of possible effects of restraint on mineral metabolism, a study was performed to directly determine if chronic restraint alters the absorption and retention of ^{45}Ca by non-anesthetized rats.

Six rats following six weeks restraint and six control rats were administered ^{45}Ca in 1-ml buffer by gavage, killed 24 hours later and the radioactivity in their femurs determined. Another 12 rats restrained for 12 weeks and 12 control rats were administered the ^{45}Ca , six by gavage and six by intraperitoneal injection. These rats were held for one week before sacrifice so that the absorption and early retention of the ^{45}Ca could be established by comparing the ^{45}Ca content of the femurs.

The results of this study are summarized in Table X. Although ^{45}Ca retention by the restrained rats was slightly lower following gavage administration, this effect was neither large nor significant. Retention of ^{45}Ca following intraperitoneal administration was the

same for the restrained and control rats. The 12 week restraint period caused a significant depression in both body and femur ash weight. Although the difference in kidney weights was not significant, both the femur ash and kidney weights were depressed in the same proportion as the body weights. However, as indicated by the percent femur ash weights, femur mineralization was not affected by the 12 week restraint period.

The number of animals in the group of rats that had been restrained for six weeks was insufficient for the depression in their body, femur ash and kidney weights to be significant. A surprising feature of these data was the, although small, significant depression in percent femur ash for the restrained rats. The reason for the significance of this small difference was that the variance of the data was reduced by dividing the femur ash weights for the individual rats by their whole femur weights. These results indicate that restraint may initially cause a depression in bone mineralization that is corrected during later growth of the rats. Blood calcium appeared not to be significantly altered by restraint, even when both chronic and acute restraint procedures were applied, Table XI.

V. Combined Effects of Restraint and X-Rays

The results of the initial voltage measurements indicated that in vitro transport measurements, like the in situ perfusion studies, would not show an effect on intestinal function from chronic restraint

Table X. Influence of Chronic Restraint on ^{45}Ca Uptake and Retention by the Rat Femur

12 Weeks Restraint

^{45}Ca in femurs (CPM) one week after gavage or intraperitoneal (I.P.) administration.

	Rats*		Probability of Difference	Degrees of Freedom
	Control	Restrained		
^{45}Ca (gavage)	5,400 \pm 400	4,600 \pm 300	>0.50	7
^{45}Ca (I.P.)	10,300 \pm 300	10,300 \pm 200	$0.50 <$	9
Femur ash wt. (g)	0.47 \pm 0.01	0.41 \pm 0.01	>0.01	22
% Femur ash	40 \pm 0.5	40 \pm 0.7	$0.50 <$	22
Kidney wt. (g)	1.56 \pm 0.04	1.44 \pm 0.04	>0.20	22
Body wt. (g)	467 \pm 8	420 \pm 8	>0.01	22

6 Weeks Restraint

^{45}Ca in femurs (CPM) 24 hours after gavage administration

	Rats		Probability of Difference	Degrees of Freedom
	Control	Restrained		
^{45}Ca (gavage)	6,700 \pm 400	6,300 \pm 1,100	>0.50	8
Femur ash wt. (g)	0.38 \pm 0.02	0.35 \pm 0.01	$0.50 <$	10
% Femur ash (g)	43 \pm 0.8	40 \pm 0.6	>0.01	10
Kidney wt. (g)	1.42 \pm 0.10	1.27 \pm 0.03	>0.50	10
Body wt. (g)	394 \pm 13	359 \pm 23	$0.50 <$	10

*The values shown are the means and their standard error. The loss of degrees of freedom from 10 to 7, 9 and 8 respectively for ^{45}Ca retention was due to loss of some of the ^{45}Ca during its administration.

Table XI. Influence of Five to Seven Weeks Chronic Restraint and Chronic Restraint Plus Acute Restraint on Blood Calcium

<u>Condition</u>	<u>Blood Calcium</u> <u>ug/ml</u>		<u>Probability</u> <u>of Difference</u>	<u>Degrees of</u> <u>Freedom</u>
	<u>Control Rats</u>	<u>Restrained Rats</u>		
Chronic Restraint	85 \pm 2	82 \pm 2	0.50<	10
Chronic Plus Acute Restraint	80 \pm 3	82 \pm 2	0.50<	10
*Acute restraint	84 \pm 2	81 \pm 2	>0.50	22

For testing the acute restraint effect, data for the control and chronically restrained rats that were not subjected to acute restraint, and the control and chronically restrained rats that were subjected to acute restraint were pooled. The values shown are the means and their standard errors.

alone. However, the marginal differences observed during the acute restraint study had been sufficient to suggest that in combination with another stress, chronic restraint might prove to have a deleterious effect on the function of the small intestine. The sensitivity of the small intestine to ionizing radiation is subject to a number of factors, of which the rate of cell turnover is one of the most important. Conditions from restraint such as altered intestinal motility, changes in the distribution of flora and in the enzyme secretions could change the rate of turnover in the intestinal mucosa without causing a significant functional difference. Therefore, it was decided to irradiate the intestine with a dose near but beneath the level that will cause death from intestinal damage; but has been demonstrated previously not to alter intestinal function in more than a small percentage of irradiated rats.

Fifteen rats were restrained for two to three weeks, fasted one day and then administered 600 R abdominal X-irradiation. On the third day after irradiation the rats were sacrificed for in vitro measurements of the bioelectric potentials and ^{22}Na , ^{36}Cl , and ^3HOH transport across the distal ileum. Measurements were also made with 15 control rats that were irradiated, six control non-irradiated rats and six chronically restrained, non-irradiated rats. Two segments of distal ileum were used from each rat; and all of the rats were fasted for four days prior to

the measurements in order to reduce differences from irradiation induced gastric restraint. The experimental procedures used for radiation exposure and the in vitro measurements are described elsewhere.⁽⁵⁾

The pertinent results from this study are summarized in Table XII. Neither chronic restraint nor prior 600 R abdominal X-irradiation had a significant effect on the bioelectric potentials or transport measurements for ^3HOH , ^{22}Na , and ^{36}Cl . There initially appeared to be an effect on the voltage measurements from irradiation, and this effect was slightly greater for the chronically restrained than control rats. However, the differences did not prove to be significant. These results are in accord with previous literature indicating that fluid and electrolyte absorption are not appreciably altered by 600 R X-irradiation.⁽⁶⁾ Although we have previously shown substantial effects on the transport and bioelectric properties of isolated ileum at higher doses of X-ray, the present results indicate that chronic restraint did not have an effect that was additive to the effect of a 600 R exposure.

Histopathological Results

Intestinal sections were taken from the rats following the in situ perfusion studies and prior to the in vitro transport measurements for histopathological examination. No effects were observed from the chronic and acute restraint conditions used. Three days after 600 R abdominal X-irradiation the intestinal villi were well populated, but the irradiated segments could be distinguished, primarily because of the presence of bizarre nuclei in the crypts (Figs. 3, 4, 5, and 6).

Table XII.* Effects of Chronic Restraint and Chronic Restraint Plus 600 R Abdominal X-Irradiation on the Bioelectric Potentials and ^3HOH , ^{22}Na , and ^{36}Cl Transport Across Isolated Ileum

Control Rats					Restrained Rats				Number of Segments and Rats
Voltage Differences (mV)	^3HOH (ul/min-segment)	<u>Na</u>	<u>Cl</u>	Voltage Differences	^3HOH	<u>Na</u>	<u>Cl</u>		
<u>Normal bicarbonate buffered media</u>									
No. Irr.	3.2			3.5					8/6
600 R	4.8			4.8					12/9
600 R	4.7	16	0.44	0.38	5.1	17	0.44	0.39	9/6
<u>Mannitol bicarbonate buffered media (Na^+, 50 mM), (Cl^-, 20 mM)</u>									
No Irr.	-4.3	13	0.72	0.85	-3.3	13	0.70	0.86	8/6
600 R	-1.2	15	0.70	0.83	0.5	13	0.66	0.82	12/9
600 R	-1.4	14	0.70	0.84	-0.7	16	0.74	0.87	9/6

*For detailed discussion of the interpretation of bioelectric and transport measurements for isolated intestine, see Ref. 4. Each of the values shown is the average for the number of segments and number of rats shown in the right hand column. The voltage differences are for the serosal surface relative to the mucosal surface of the intestinal segments. The intestinal segments were everted and two cm in length. The values for tritiated water are the unidirectional mucosal to serosal fluxes divided by the concentrations of tritiated water in the mucosal media. These values have the dimensions of permeability and are termed the apparent absorption permeabilities. For ^{22}Na and ^{36}Cl , the absorption permeabilities for each segment were divided by the respective absorption permeabilities for tritiated water. These values are unitless and are designated as Na and Cl in the Table. (Continued on next page).

Table XII continued:

All of the segments were first placed into the normal bicarbonate buffered media for voltage measurements, and transport measurements were made for segments from six of the control rats and six of the chronically restrained rats that had been irradiated. All of the intestinal segments were then transferred into the mannitol bicarbonate buffered media for further voltage and transport measurements. As shown in the mannitol media the serosal surface of the segments was usually negative with respect to the mucosal surface, and the absorption permeabilities for Na and Cl increased to about 70 and 90 percent of those for tritiated water respectively. For seriously damaged tissue, these effects would not have occurred. During restraint the average weights for 15 of the chronically restrained rats (data for the 8 non-irradiated and 12 irradiated intestinal segments in Table XII) increased from 307 to 310 grams and for the comparable control rats 312 to 329 grams. For the other six restrained rats, their average weight increase was 347 to 357 grams, and the six control rats, 346 to 371 grams.

Discussion

The results of this study did not indicate that chronic restraint of body movement had a serious effect on intestinal function. The absence of a significant effect on the ileum from combined chronic restraint and 600 R abdominal X-irradiation is particularly significant, because most deleterious effects would probably alter the rates of sloughing and renewal of cells in the intestinal villi.

Both the results obtained by Pfeiffer⁽²⁾ and the present data demonstrate that chronic restraint retards the growth of rats. These results indicate, however, that the effect is not a direct effect on the absorptive cells of the small intestine to transport the essential nutrients studied. It is quite probable that other gastrointestinal functions such as gastric emptying, intestinal motility, or defecation, which are more dependent upon associate body movement, are adversely affected by the lack of exercise. Research should be performed in that area to determine how seriously these functions are affected by restraint. Other studies should also be performed on adrenal and thyroid function in restrained animals, and on their ability to withstand cold. Information of that nature would have both basic and practical value.

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FIGURE LEGENDS

- Figure 1 Metabolism cages for restrained (A) and control rats (B).
- Figure 2 Bioelectric potential differences across isolated rat intestine. Because of the absence of a significant effect from the two weeks of chronic restraint, data from the restrained and control rats were pooled. The values shown are the averages for 28 jejunal (usually 3 segments per rat), 20 anterior ileal (2 segments per rat) and ten distal segments (1 segment per rat). The rats were fasted one day and then fed two hours before the experiments were initiated. The segments were first placed into a normal bicarbonate buffered media, then transferred to a bicarbonate buffered media with the Na^+ concentration reduced to 20 mM by choline chloride substitution for NaCl, and then transferred back to the normal bicarbonate buffered media.
- Figure 3 Control Ileum. Normal villi with intact surface epithelium and goblet cells. The crypts show active mitotic activity and there are a normal number of cells in the villus core.

Figure 4 Chronic Restraint. No irradiation. The section here is at right angles to the control Fig. 1 so that the lamina propria and core of the villus do not appear to be as wide. The surface epithelial cells, however, show no changes and the crypts are of normal height and show active mitotic division.

Figure 5 Three days after 600 R abdominal irradiation. No restraint. There is a marked reduction in the number of surface epithelial cells and their nuclei are increased in size. They have a coarse nuclear chromatin pattern with some being pyknotic. The cells show some cytoplasmic vacuolization and the contents of the goblet cells are tense and bulging. In the crypts the cells are hyperchromatic but mitotic activity can be seen near the base of the crypts and no crypt dilatation is present.

Figure 6 600 R abdominal irradiation plus chronic restraint. The changes here are similar to those in Fig. 3. The irradiation effects are perhaps better illustrated here yet active mitotic division can be seen in the crypts.

